Following nanomedicine activation with magnetic resonance imaging

Reessing, Friederike; Szymanski, Wiktor

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Following nanomedicine activation with magnetic resonance imaging: why, how, and what's next?

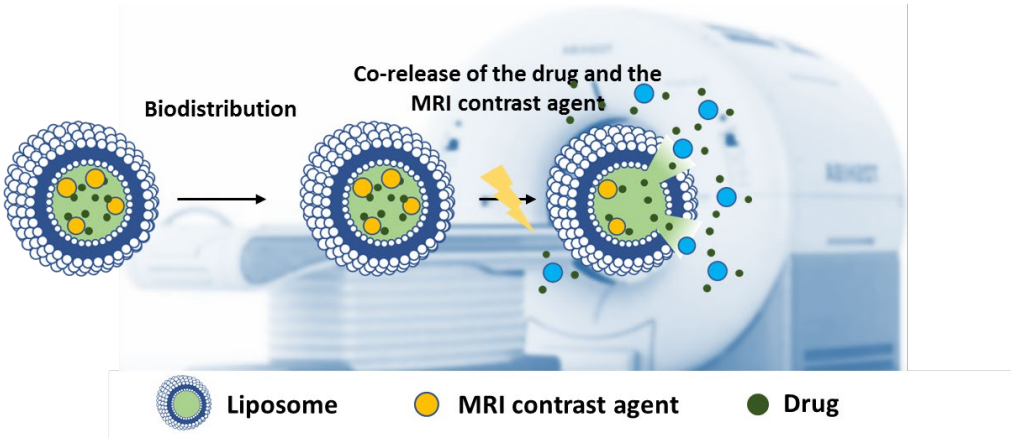
Friederike Reeβing[1,2] and Wiktor Szymanski*[,1,2]

[1] University Medical Center Groningen, Department of Radiology, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

[2] Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty of Science and Engineering, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

* Corresponding author: Szymanski, Wiktor (w.szymanski@umcg.nl)

Graphical abstract



Abstract

Nanomedicines, such as liposomal formulations, play an important role in cancer therapy. To support their development, medical imaging modalities are employed for following the drug delivery. Encapsulation of MRI contrast agents, which change their relaxivity upon co-release with the drug, is a promising strategy for monitoring both the biodistribution and payload release from a nanocarrier. This approach is successfully applied in preclinical settings to image the activation of liposomes responsive to heat, pH changes or sonication. Recent advances include combination with different treatments and the implementation of chemical exchange saturation transfer imaging to gain spectral resolution over different contrast agents. However, this field still faces challenges, such as matching the pharmacokinetic profiles of the contrast agents and the liberated drugs.

Introduction

Cancer nanomedicine continues to hold great promise for targeted chemotherapy that limits harmful side effects while enhancing drug solubility and circulation time [1]. It relies mostly on the use of lipid- or polymer-based nano-sized carriers filled with a cytotoxic payload that is delivered and released in the tumor [2]. Drugs that are approved as liposomal preparations include for instance doxorubicin, paclitaxel and vincrisitine, with numerous others being in advanced phases of clinical trials [3].

The basic principle behind the tumor-selectivity of nanomedicines is the enhanced permeability and retention (EPR) effect, whereby the leaky vasculature and decreased lymphatic drainage result in the accumulation of the drug in the tumor. However, the selectivity can be further enhanced through the use of active targeting moieties (e.g. folate or antibodies)[4] or control over the site of payload release, using endogenous (e.g. pH or enzymes) or exogenous (e.g. light, heat, ultrasound) triggers [5].

In the efforts to capitalize on the promise of triggered drug delivery in nanomedicine, the development of medical imaging techniques that enable studying the distribution and drug release *in vivo* is of key importance [6]. Here, Magnetic Resonance Imaging (MRI) is often the modality of choice, as it offers high resolution with minimal invasiveness and no radiation burden [7,8].

Incorporation of MRI contrast agents, usually Gd^{3+} complexes, into nanomedicines (Figure 1a) enables their tracking inside the patient body and confirmation of the localization in the tumors. However, it provides no information on the efficiency and location of drug release, which depends on many factors. For endogeneous triggers, it is not certain if the lower pH or increased enzymatic activity in the heterogeneous tumor environment are pronounced enough to act as triggers. For exogeneous stimuli, the attenuation of the signal in the tissue (in case of ultrasound and light) and the inefficient heat transfer (in hyperthermia) may affect the dose of the trigger needed for efficient cargo release.

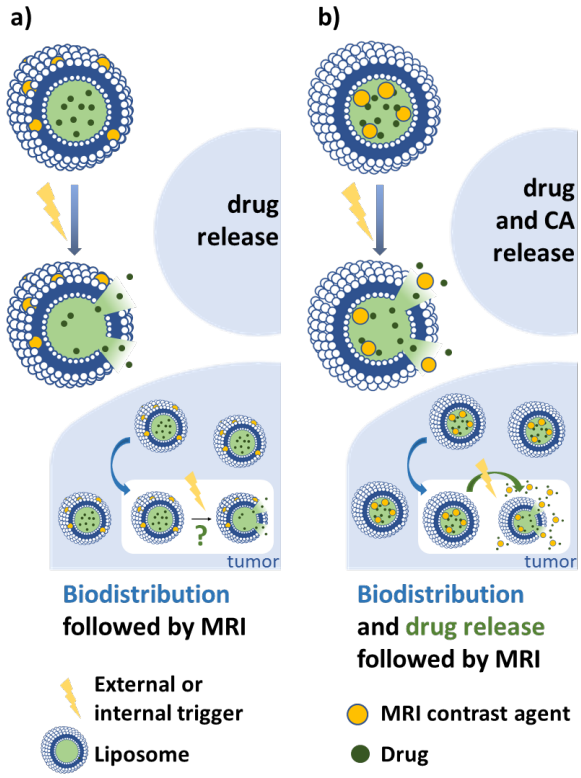


Figure 1. Approaches to MRI guided liposomal drug delivery. a) Incorporation of a CA in the bilayer allows following of the biodistribution of the drug carrier but does not provide information about drug release. b) Loading of the CA inside the carrier enables monitoring the cargo release.

In this Opinion, we critically review approaches that are aimed at MR imaging of not only the nanocarrier distribution, but also the payload release (Figure 1b). The most commonly used strategy is the co-encapsulation of the drug and the contrast agent (CA) inside the lumen of the carrier (for an overview of the published systems and their characteristics, see Table 1). MRI CAs usually operate on the principle of shortening the relaxation time of surrounding water protons. When they are encapsulated in the liposome, their action is impaired due to the limited diffusion of water through the lipid bilayer. Once the CA is released, enhanced water exchange leads to an increase of relaxivity that can be measured in an MRI scan. The following sections describe the main applications of MRI in monitoring the payload release from nanomedicines triggered by heat, ultrasound and low pH, followed by a critical discussion and outlook on future directions for the field.

Table 1. Overview of nanomedicines that incorporate MRI contrast agents for imaging of the payload release

Ref	Trigger	Contrast agent[a]	MRI modality	Change in relaxivity after triggering	Nanocarrier composition	Drug	Test system
[9–11]	Heat, hot water catheter	Mn^{2+}	T_1	ΔT_1 of 30-40%	Liposomes: DPPC/MSPC/DSPE-PEG200, 90:10:4	Doxorubicin	Rats with fibrosarcoma
[12]	Heat, IR laser	Gd-DTPA-BMA	T_1 (0.47 T, 1.5 T)	ΔR_1 of 0.70±0.06 $mM s^{-1}$	Liposomes: DSPC/DSPG, 95:5	None	Rabbit
[13, 14]	Heat, water bath	Gd-DTPA-BMA	T_1 (0.5 T, 1.5 T)	R_1 change from 0.4 to 4.2 $mM s^{-1}$	Liposomes: DPPC:DSPC:DPPG$_2$, 50/20/30	None	Mice with BFS-1 tumor
[15]	Heat, hot water bath	Gd-DTPA	T_1 (7 T)	60% reduction in T_1	Liposomes: DPPC/Brij78, 96:4	Doxorubicin	Mice with EMT-6 tumor
[16, 17]	Heat, IR laser	Different Gd complexes	T_1 (0.47 T, 3 T)	T_1 decrease up to >6x, temperature dependent (*in vitro*), correlation with drug release (*in vivo*)	Liposomes: DPPC/DSPC/DPPG$_2$, 50:20:30	Doxorubicin (in different liposomes)	Rats with BN175 tumors
[18]	Heat, HIFU	Gd-HP-DO3A	T_1 (0.5 T)	R_1 change from 1.95 to 4.01 $mM s^{-1}$	Liposomes: DPPC/MSPC/DSPE-PEG2000,	Doxorubicin	Rabbit VX2 tumor model

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[19, 20]	Heat, HIFU	Gd-HP-DO3A	T_1, T_2 (3 T, 7 T)	T_1 ~5x decrease, T_2 ~3x decrease	Liposomes: DPPC/HSPC/Chol /DPPE-PEG2000, 50:25:15:3	Doxorubicin	Rats with 9L tumor
[21 – 23]	Heat, HIFU	Gd-HP-DO3A	T_1	ΔR_1 up to 0.12 mMs^{-1}	Liposomes with ^{111}In: DPPC/HSPC/Chol /DPPE-PEG2000/DOTA-DSPE, 50:25:15:3:1	Doxorubicin (+ ablation treatment)	Rats with 9L and rhabdomyosarcoma tumors
[24]	Heat, HIFU	Gd-BOPTA	T_1 (4.7 T, 7 T)	R_1 change from 1.32 (37 °C) to 6.64 mMs^{-1} (42 °C) (*in vitro*). ΔR_1 of 0.13 *in vivo*	Liposomes: DSPE/DSPC/DSPE -PEG2000/Chol, 41:14:2:10	Doxorubicin	Mice with SCC-7 tumor
[25]	Heat, HIFU	Gd-HP-DO3A	T_1 (3T)	ΔR_1 up to 0.7 mMs^{-1}, correlated with drug release	Liposomes: DPPC/DSPC/chol /DPPE-PEG2000, 53:22:15:3	Doxorubicin	Rats with 9L tumor
[26]	Heat, HIFU	Fe-SDFO	T_1 (3T)	R_1 change from 0.8 to 1.35 mMs^{-1}	Liposomes: DPPC/DSPC/chol /DPPE-PEG2000, 61:14:15:3	Doxorubicin	Rats with 9L tumor
[27]	Heat	Mn^{2+} and Gd-HP-DO3A	T_1 (NMRD profiles)	Mn: R_1 change from 5.1 to 32.2 mMs^{-1} Gd: R_1 change from 1.2 to 4.4 mMs^{-1}	Liposomes: DPPC/MPPC/ /DPPE-PEG2000, 86:10:4	Doxorubicin	*in vitro*
[28]	pLINFU	Gd-HP-DO3A	T_1 (7 T)	35-40% of T_1 contrast enhance	Liposomes: DPPC/DSPE-PEG2000, 95:5	none	Mice with B16 melanoma
[29, 30]	pLINFU	Gd-HP-DO3A	T_1 (7 T)	T_1 contrast to noise ratio increase ~4 times	Liposomes: DPPC/DSPC/Chol /DSPE-PEG2000, 10:5:4:1	Doxorubicin	Murine TS/A breast cancer model
[31]	pLINF	Eu(HPD	paraCES	Contrast	Liposomes:	None	Phantom

	U	O3A)	T	switched on	DSPC/DSPE-PEG2000, 95:5		
[31]	pH	Tm(HP DO3A)	paraCES T	Contrast switched on	Liposomes: POPE/tocopherol hemisuccinate/chol 44:12:44	None	phantom
[32]	pH	Gd-HP-DO3A and others	T_1 (0.47 T, 7 T)	R_1 changed ~3x when pH lowered from 7.2 to 5.5	Liposomes: POPE/THS/Chol (4:1:4)	None	*In vitro*
[33]	pH	Mn^{2+}	T_1, T_2 (9.4 T)	R_1 increase 12.5 x R_2 decrease 9.6 x	Liposomes loaded with As-Mn complex	Arsenic trioxide	GBM16 and GBM44 cells *in vitro*
[34]	pH	Fe nanoparticles	T_2 (3 T)	T_2 change >9 x	FePt nanoparticles on graphene oxide conjugated with folate	Toxic Fe ions that catalyse ROS formation	Mice with 4T1 tumour model

[a)] Abbreviations used: Gd-DTPA-BMA: Gadodiamide, Gd-DTPA: Gadopentetate dimeglumine, Gd-HP-DO3A: Gadoteridol, Gd-BOPTA: Gadobenate dimeglumine, Fe-SDFO: Fe-succinyl deferoxamine, T_1: longitudinal relaxation time, T_2: transverse relaxation time.

Heat-triggered drug release from thermosensitive liposomes (TSL)

Local induction of hyperthermia is well established to trigger the targeted release of drugs from nano-carriers [35,36]. This approach employs, as a vehicle for drug delivery, thermosensitive liposomes, whose membrane becomes permeable at a certain temperature. TSLs are prepared by adjusting the phase transition temperature (T_m) of the liposomal membrane to 39 – 42 °C, through the incorporation of e.g. DPPC (Dipalmitoylphosphatidylcholine) into the bilayer. The T_m needs to be tuned in such a way that the liposomes are sufficiently stable at normal temperature but show efficient cargo release upon heating [19]. At temperatures around the T_m, the lipids of the bilayer coexist in both the solid gel phase and the liquid-crystalline phase, leading to defects in the membrane and increased permeability [35]. These characteristics can be further improved by expanding the lipid composition with cholesterol [24], lysolipids [36] or $DPPG_2$ [13,14,16,17].

Heat treatment does not only trigger the targeted release of drugs from liposomes but is also known to enhance the antitumor effect [37], due to enhanced permeability and blood circulation, among others. It has to be noted that, in contrast to conventional liposomal drug delivery systems, the payload of TSL is generally not delivered in an intracellular but intravascular fashion, before the liposome is taken up. The various ways of heat delivery to the target tissue used in *in vivo* studies include immersing body parts in a warm water bath, insertion of heated catheters and the use of IR

lasers, microwave irradiation or high intensity focused ultrasound (HIFU).

MR thermometry is regularly used, in particular for monitoring hyperthermia induced by HIFU, and dedicated hybrid systems are already established [38,39]. However, in the conventional setups, MRI only provides information about the local temperature, but not about the content release from TSL. Therefore, loading the TSL with a paramagnetic CA that is liberated together with the cargo when the T_m is reached has been investigated. The underlying concept has already been proven *in vivo* in the early 2000, using either Mn^{2+} or Gd^{3+}-complexes as CA for T_1 weighted imaging [9,12]. In the following years, the respective systems have been optimized and analyzed in depth, regarding the release kinetics [18], the correlation between CA and drug liberation (Figure 3a-c) [15,20], and the influence of the time difference between the liposome application and hyperthermia treatment [11] (Table 1). Moreover, the combination of heat-induced release of doxorubicin for tumor treatment and thermal ablation has been assessed, showing that the shutdown of vasculature due to ablation treatment impairs the effectiveness of drug delivery and therefore should be applied after triggering the drug release [23]. Another report addresses the challenge of simultaneous MR thermometry and T_1 imaging for monitoring of CA release by introducing an interleaved scan protocol [25]. Besides manganese and gadolinium-based CAs, also an iron-complex was evaluated as a CA and the respective advantages and disadvantages were evaluated, as described later in this Opinion. Moreover, the various examples of TSL differ in their (phospho-)lipid composition and the corresponding T_m of the liposome membrane [16,19]. Some reports also asses the possibility of multimodal imaging, employing an ^{111}In-complex integrated in the bilayer of the TSL. In such applications, the release of the MRI CA can be observed not only by a change in relaxivity but also by the loss of co-localization of the SPECT/CT and MRI signal. The exact distribution of doxorubicin and liposomal phospholipids can be analyzed by fluorescence imaging and autoradiography respectively [21–23].

In summary, MR imaging of thermally induced content release became an established method in preclinical research and has high chances to enter the clinical stage soon. Recent publications focus on the practical execution of the MRI scan. Even though the setups for HIFU treatment under MR guidance are available, the simultaneous MR thermometry and analysis of contrast agent release remains challenging and is addressed in recent reports [25].

pLINFU-triggered drug release from sonosensitive liposomes

As opposed to HIFU, which is used to locally increase the temperature and enable payload release from thermosensitive liposomes (see the section on heat-triggered drug release), the triggered delivery of the cargo using pulsed low intensity non-focused ultrasound (pLINFU) relies on mechanical interaction of the nanocontainer with acoustic waves [40,41]. The use of low energy US limits the cytotoxicity related to cavitation effects and heating, and enables the activation of non-thermosensitive nanocontainers [29]. The usefulness of pLINFU in drug delivery has been demonstrated *in vitro* [42] and in in a pre-clinical setting [43,44].

In a seminal series of publications [28–30], Rizzitelli, Terreno and co-workers evaluated the use of MRI to study the pLINFU-induced co-release of doxorubicin and gadoteridol from liposomes *in vivo*. Release of the material resulted in T_1 signal increase of 35-40% in the tumor in a mouse model [28]. In a follow-up study [29], a hybrid protocol was established, in which contrast-enhanced MRI was used to guide the payload release and morphological MRI enabled the monitoring of the therapeutic effect. Two key aspects of the system were studied: i) using relaxometry (gadoteridol) and spectrofluorimetry (doxorubicin), the co-release of both compounds was confirmed *in vitro*, thereby showing that pLINFU does not only increase the water diffusion through the liposome membrane but also enables the drug release; ii) liposomes were found to play a crucial role in the permeabilisation of the vascular endothelium by pLINFU, likely acting as acoustic resonators. Finally, the same group proposed [30] the use of two sequential pulsed US stimuli: one to trigger the release of the drug and the other to increase the tumor vascular permeability (sonoporation) and enable the drug diffusion to the stroma, leading to an almost complete tumor regression in a breast cancer mouse model.

A different strategy for visualizing the payload release from liposomes was presented by Delli Castelli, Aime and co-workers (Figure 3d) [31]. It is based on chemical exchange saturation transfer (CEST) effect, an emerging MR imaging modality. In CEST [45], the localization of molecules that contain labile protons with a specific chemical shift can be visualized, through subsequent selective magnetic saturation of those protons, allowing their exchange with the pool of water protons, and voxel-by-voxel imaging of the saturation. In paraCEST, lanthanide complexes are used that contain water-exchangeable protons with chemical shifts that fall outside the range of typical values and can therefore be selectively addressed. When paraCEST agents are loaded into liposomes, the exchange of protons with bulk water molecules is limited, which quenches the CEST signal. Payload release, triggered by insonation, resulted in increased signal. Additionally, lipoCEST effect could be used in parallel to visualize drug delivery, using the chemical shift of the intraliposomal water protons.

pH-triggered drug release from acid sensitive liposomes

The use of increased acidity of the environment as a trigger for drug release from nanocarriers is inspired by two effects. Firstly, the interstitial pH in tumors is known to be lower than in healthy tissue [46], enabling selective delivery. Secondly, once the liposomes are taken up into the cell, the release can be achieved due to higher acidity in the endosomal/lysosomal system [47]. The formulation of pH-responsive liposomes is based on combinations of lipids that undergo phase transition under acidic condition, usually based on phosphoethanolamine and anionic amphiphiles with basic sites [48].

The model studies on the release of MRI contrast agent gadodiamide from pH-responsive liposomes were described by Løkling *et al.* in a series of papers focusing on *in vitro* studies [49] and optimization [50,51] of MR properties. Those studies were followed by the report from Torres, Terreno and co-workers [32], who evaluated different gadolinium-based CAs and studied the co-release of the CA and a model fluorescent compound at different pH (Figure 2c).

A conceptually different approach to pH-triggered theranostics nanomaterials is based on systems in which the cytotoxic material, or a precursor thereof, itself exhibits MR properties that enable

imaging. Zhang, Zhao and co-workers presented [33] liposomes that nano-encapsulate arsenite-manganese precipitates, in which As^{3+} ions are used for therapy and Mn^{2+} ions enable T_1- and T_2-weighted MR imaging. After being taken up into cells, the liposomes release the ions in response to acidic environment in endosomes/lysosomes, resulting in an increased T_1 signal. At the same time, the T_2 signal decreases due to the dissolution of precipitates that possess magnetic susceptibility.

T_2-weighted imaging can also be used to study the release of toxic iron ions from iron nanoparticles (FeNPs), as shown by Yue, Yu and co-workers [34] for a nanoassembly comprising FeNPs and graphene oxide functionalized with folic acid for tumor targeting. Also here, the endosomal/lysosomal release of iron was envisioned, followed by Fe-catalyzed formation of reactive oxygen species from hydrogen peroxide produced by mitochondria. A slow decrease in T_2 signal was observed in the tumor region in a mouse model, indicative for toxic cargo release.

The CEST imaging approach to study drug delivery from liposomes, used in the pLINFU-triggered approach (see the section on pLINFU-triggered drug release) has been extended also to pH-responsive systems (Figure 3d) [31]. The use of phosphoethanolamine-derived lipids enabled the cargo delivery at pH<6. Interestingly, the use of two different lanthanides (Eu and Tm), in sono- and pH-responsive liposomes respectively, enabled the study of release from both nanosystems in parallel. This was possible due to the different chemical shift of water in the metal complexes, which facilitates selective addressing of them with the saturation pulse.

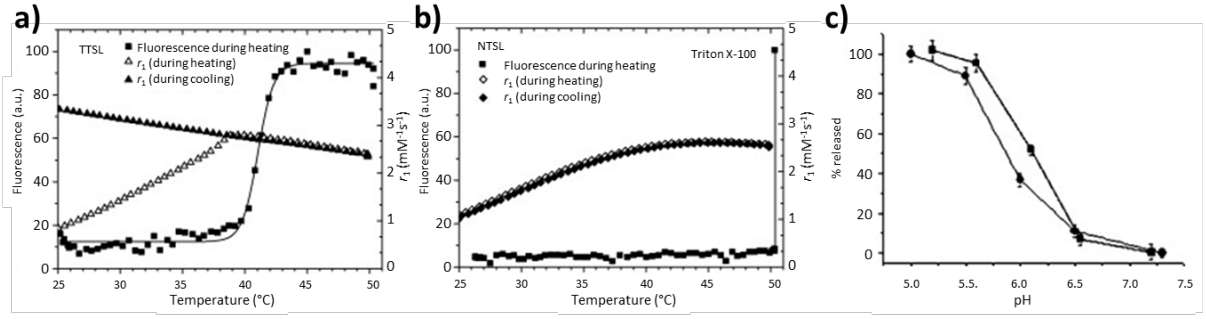

Figure 2. Studies on the release of cargo from heat- (a and b) and pH-responsive (c) liposomes. Changes in T_1 relaxivity (r_1) and fluorescence during heating and subsequent cooling of traditional temperature sensitive liposomes (TTSL, a) and non-temperature sensitive liposomes (NTSL, b). Increased fluorescence indicates the release of doxorubicin, since its dilution leads to dequenching. a) The irreversible increase in relaxivity and increase in fluorescence shows the release of the CA and doxorubicin. b) The reversible relaxivity increase stems from enhanced water permeability of the liposomal bilayer at elevated temperature. No increase in fluorescence is observed during heating, indicating that no doxorubicin is released. Addition of a detergent (Triton X) to destroy the liposomes leads to an increase in fluorescence, proving that the liposomes stayed intact during the hyperthermia treatment. Panels a and b adapted with permission from ref. [19] Copyright 2010, Elsevier. c) Fraction of CA and model compound (carboxyfluorescein) release at different pH from pH-sensitive liposomes, calculated by increase in relaxivity (squares) and fluorescence (circles). Panel c adapted with permission from ref. [32] Copyright 2011, Elsevier.

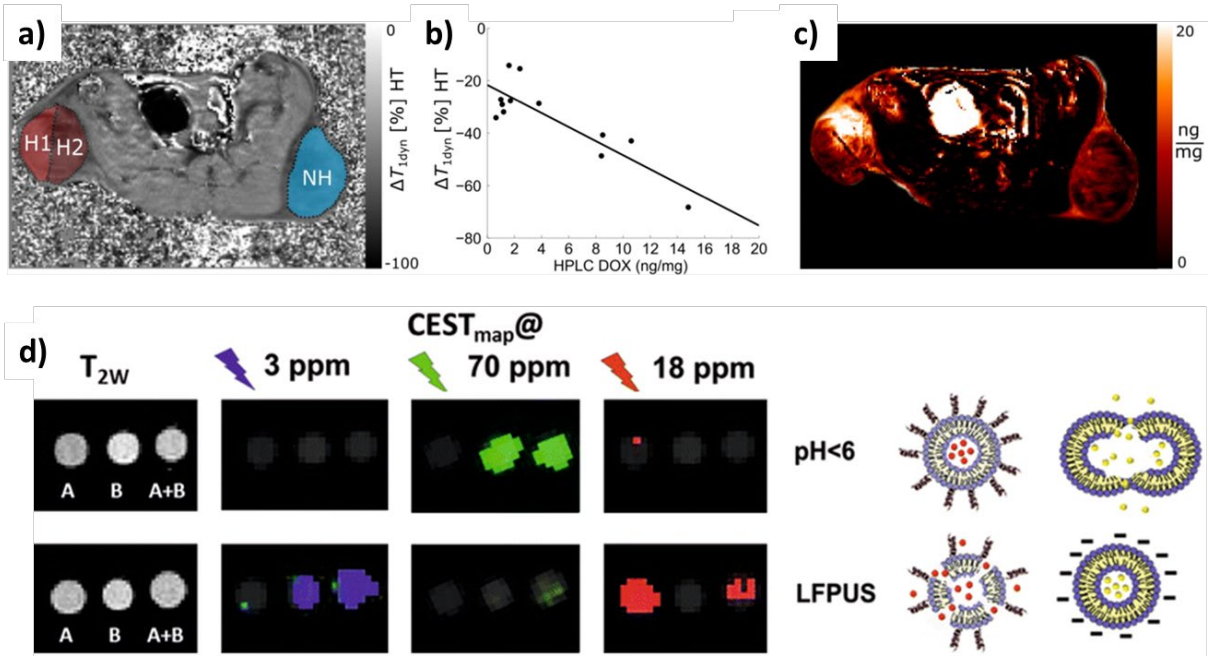

Figure 3. MR and CEST imaging of temperature- (a-c), pLINFU- (d) and pH-triggered (d) release of content from liposomes. a) Cross-section of a rat in supine position with two tumors, overlaid with ΔT_{1dyn} map, which shows the difference between the T_1 signal before injection and at the end of hyperthermia (H1 and H2 indicate the tumor exposed to heat; NH is the non-heated tumor); b) Linear regression of doxorubicin concentration (measured by HPLC) with ΔT_{1dyn}; c) Doxorubicin concentration map calculated from (a) using regression from (b), selective release of doxorubicin in heated tumor is visible; Panels a-c adapted with permission from ref. [17] Copyright 2016, Elsevier. d) T_2-weighted and CEST map images of a phantom containing liposomes A (pLINFU-sensitive, loaded with Eu-based CEST agent with saturation offset of 18 ppm), liposomes B (pH-sensitive, loaded with Tm-based CEST agent with saturation offset of 70 ppm) and a mixture thereof. Selective imaging is possible when low pH (upper row) or ultrasound (lower row) is applied. Panel d adapted with permission from ref. [31]. Copyright 2014 Springer.

Discussion

The various reports described in this Opinion support the feasibility of MRI-monitored liposomal drug delivery that rely on different stimuli to trigger drug release. Nonetheless, this field is still facing various challenges and there are a few essential topics for consideration. First of all, the basis of the approach lies in the assumption that the CA and the drug are released simultaneously. This certainly has to be proven for each individual case, since it depends on the physicochemical properties of the two compounds [29]. These properties should therefore be as similar as possible to guarantee simultaneous release. Thus, the choice of the CA has a crucial influence on the imaging outcome. Besides the release kinetics, also the distribution and metabolism/excretion should ideally coincide. Since these characteristics are different for each compound, there is no ideal CA; instead, depending on the co-encapsulated drug, the best combination has to be established. Gadolinium complexes, for instance, are normally cleared rapidly from the delivery site and tend to accumulate in the liver and

spleen. Conversely, manganese ions are often retained at their point of release owing to their interaction with phospholipids [27]. Next to gadolinium and manganese, also the use of iron-based contrast agents has been suggested, offering the advantage of higher biocompatibility [26]. However, the low T_1 relaxivity compared to the other two types of CA restricts the application as it imposes the use of very high concentrations of the iron complexes.

The incorporation of high quantities of CAs into the liposomes is limited by the resulting osmotic pressure. In this respect, the use of uncharged gadolinium complexes (such as Gd-HP-DO3A or Gd-DTPA-BMA) is beneficial, as they can be loaded in a higher concentration resulting in a higher change in relaxivity before and after release [16]. However, linear uncharged complexes are known to be less stable and hence bear a higher risk to release free Gd^{3+} ions causing severe side effects [52]. One strategy to increase the concentration of the CA in the liposomes is to administer the CA and the drug independently in two different liposomes [26]. Besides boosting the relaxivity change, this approach opens the possibility to deliver two incompatible compounds, such as an iron-complex as CA requiring acidic pH and doxorubicin as cytotoxic agent. However, it gives less control over the actual co-release of CA and drug and renders the system less reliable, especially in a complex system such as the human body.

In general, it is not only crucial to confirm the co-release of the drug and the CA, but also to prove that the increase in relaxivity in fact stems from the release of the CA. As explained above, the relaxivity of the encapsulated CA is relatively low due to limited water exchange. Thus, a higher water permeability of the liposomal membrane enhances the relaxation rate without actual release of the CA (Figure 4a) [9,12]. This way, for example mild hyperthermia below the T_m increases the water permeability and can induce a signal increase, erroneously indicating drug release. In contrast to signal enhancement by CA release, the relaxivity change is reversible, which can readily be analyzed in *in vitro* experiments to exclude the false positive result as exemplified in figure 2a-b [19]. However, the respective experiments do not provide information about the exact mechanism of CA release and do not answer the question if the liposomal carrier stays intact after payload delivery. Studies using radiolabelled lipids indicate the accumulation of empty liposomes in liver and spleen and thus suggest that their cargo is released via transiently formed pores [22].

Another point to consider is the chronological setup of the treatment [11]. Especially when external triggers, such as heat, are applied to induce cargo release from the liposomes, the initiation of release and acquisition of MR signal have to be timed carefully. This is even more important when the pharmacokinetic profiles of the CA and the drug after release differ. The combination with other treatments or imaging modalities represents an additional practical challenge [25].

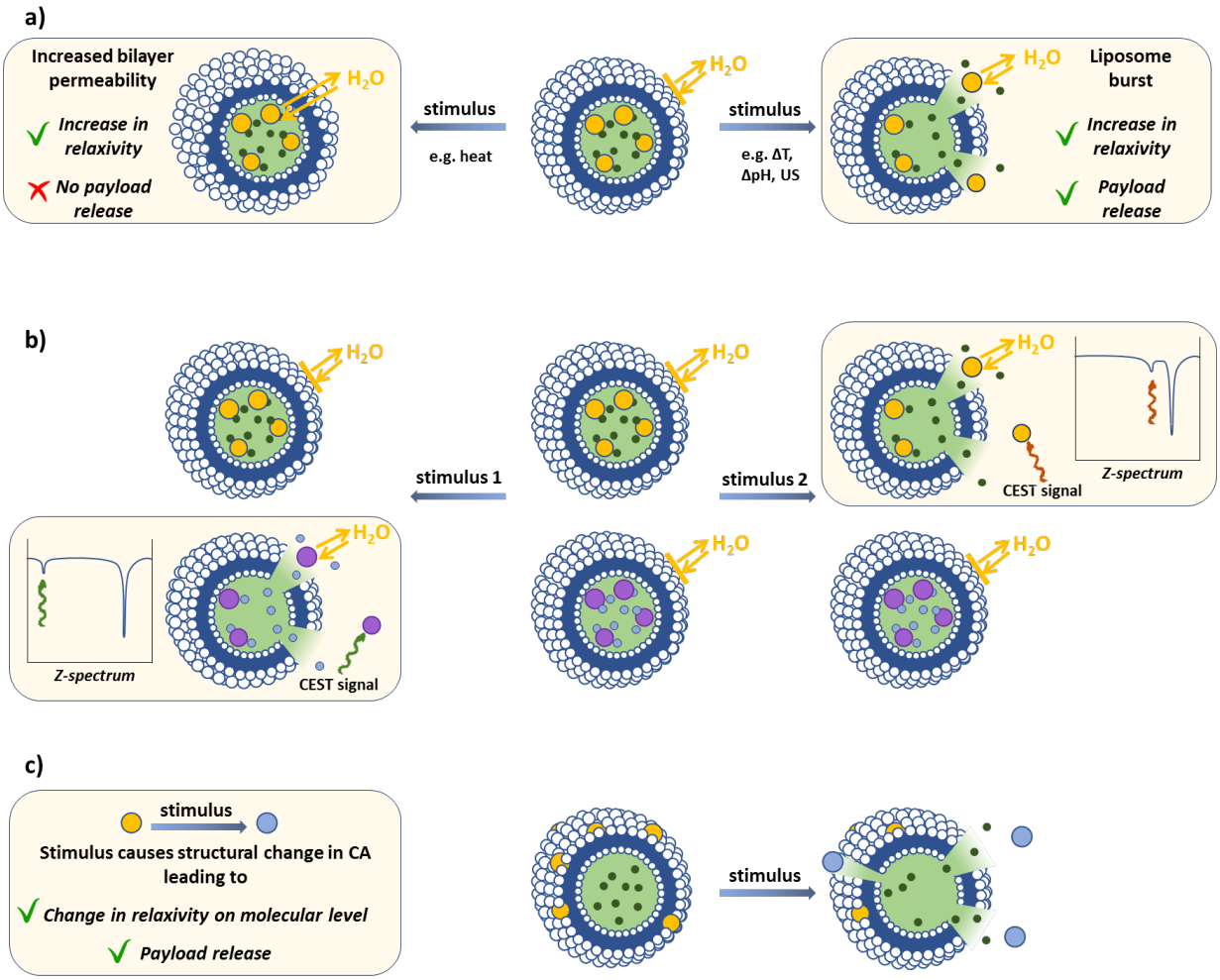

Figure 4. Consideration points and outlook for the use of MRI in studying the activation of triggered nanomedicines. a) Relaxivity increase can stem from the release of the CA with simultaneous drug release or only from increased water exchange due to higher permeability of the bilayer. The latter is reversible and does not coincide with cargo release. b) Monitoring of orthogonal payload release using various liposomes responsive to different stimuli is possible with CEST imaging due to spectral resolution of the distinct chemical shifts of the different CEST contrast agents. c) The incorporation of a stimuli-responsive contrast agent into the liposomal bilayer, causing disintegration and cargo release from the carrier upon a trigger, allows the unambiguous confirmation that the trigger has reached the liposome and is not sensitive to spontaneous cargo release.

Conclusion and Outlook

With the proof-of-principle studies in place, and numerous *in vivo* reports emerging, the use of MRI to follow the release of drugs from nano-carriers gains momentum and is set to develop into hospital practice. While the nanomedicine field has been argued to under-deliver on its enormous promise [6], we believe that methods that allow the assessment of the location and efficiency of drug release will provide the boost for clinical translation.

To achieve this, further development of CAs with various properties, which are as similar to those of the active substances used nano-carriers, is required. Currently the model applications rely almost exclusively on the use of doxorubicin (see Table 1) as a drug, but numerous other drug preparations are being approved for clinical use, which would require different CAs to follow. Considering the recent concerns on the accumulation of gadolinium in human tissues after CA-enhanced MRI scans [53], which led to legal actions prohibiting the use of most linear gadolinium complexes [54], this becomes an important challenge that needs to be met if the use of MRI to follow the distribution and activation of nanomedicines is to find a general use in clinics.

The field would further benefit from the establishment of multifunctional membrane constituents that not only bear a contrast enhancing moiety but are also susceptible to a trigger resulting in cargo release. This way the imaging of nano-carrier activation would be enabled, conveying a higher certainty about accordance of the signal increase with liposome responsiveness (Figure 4c).

Finally, we also highlight here the possibilities offered by the selective delivery of different drugs induced by orthogonal stimuli. Since the T_1 and T_2 relaxivity measurement offer practically no spectral resolution, new MRI modalities are needed for this purpose. The emerging CEST imaging (Figure 3d) fulfills this requirement, as it enables the imaging of separate CA independently of each other due to their distinct chemical shift (Figure 4b).

With the increasing understanding of the intricacies of the drug/CA release from nanomedicines, a set of guidelines for the field emerges. In this opinion, we aimed at highlighting the opportunities, challenges and pitfalls, hopefully providing an outlook for the future development towards clinical practice.

Acknowlegments:

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